library has substantially no insert size bias relative to a population of genomic DNA or cDNA molecules used in making said library.

64. A genomic or cDNA library in a circular vector, wherein said genomic or cDNA library has substantially no insert size bias relative to a population of genomic DNA or cDNA molecules used in making said library.

65. The mixture of Claim 33, wherein said circularized vectors do not comprise a bacteriophage cos site.

66. The linearized vector of claim 37 or 38, wherein said cohesive circularization ends do not comprise cohesive ends of a bacteriophage.--

REMARKS

Applicant has submitted an Information Disclosure Statement with this Response and requests consideration thereof. Applicant has also amended the claims and specification to clarify the language therein and to correct inadvertent typographic errors. Applicant submits that these minor changes add no new matter to the specification.

Sequence Listing

As requested by the Examiner, Applicant has amended the specification to correct inadvertent typographic errors in the Sequence Listing. Applicant has also provided a substitute computer readable form of the Sequence Listing and a statement verifying that the content of the paper and computer readable copies are the same. Applicant submits that the Sequence Listing complies with the requirements of 37 C.F.R. §§1.822 and 1.823 and that the application is in condition for allowance, which action is earnestly solicited.

Drawings

The Patent Office has objected to the drawings as allegedly having improper margins and lettering. Applicant respectfully submits that these corrections will be made and final drawings will be submitted upon allowance of the application.

Claim Objections and Rejections Under 35 U.S.C. § 112

The Examiner has objected to certain typographical errors in Claims 17 and 23. These claims have been amended to correct these inadvertent typographic errors.

The Examiner has also rejected Claims 22 and 23 as allegedly indefinite under 35 U.S.C. § 112, second paragraph. The Examiner has alleged that the term "selected from the group consisting essentially of" in Claim 22 is indefinite through use of "essentially," which according to the Examiner makes definition of the group unclear. "Essentially" has been deleted from Claim 22. The Examiner has alleged that the term "said first nucleic acid concentration can comprise about" in Claim 23 is indefinite because it is unclear whether or not the first acid concentration comprises the stated concentration and because 10^{-21} to about 10^{-14} is an amount, rather than a concentration. Claim 23 has been amended to clarify the language therein. Applicant submits that Claims 17, 22 and 23 are definite and requests withdrawal of the rejection under 35 U.S.C. § 112, second paragraph.

Claim Rejections Under 35 U.S.C. § 102

Claims 31-32 have been rejected under 35 U.S.C. § 102(e) as alleged anticipated by U.S. Patent 5,844,084 to Guegler. The Examiner alleges that Claims 31-32 are drawn to a nucleic acid insert and a cDNA library in a circular vector and that Guegler anticipates these claims because it discloses a circular vector containing a nucleic acid insert. According to the Examiner, any novelty for Claims 31 and 32 resides in the method of

making these products.

Claims 31 and 32 have been canceled without prejudice. Product Claims 51-64, containing some of the subject matter of Claims 31-32 have been added. In general, the subject matter of Claims 51-64 are directed to linear and circular nucleic acid insert-vector constructs and genomic or cDNA libraries. These constructs and libraries have distinct circularization ends which are not found in Guegler. The circularization ends of Claims 51, 52, 57 and 58 are attached to an enzyme or enzyme complex capable of covalently joining those cicularization ends, and those ends are at least about 20 base pairs from each end of the insert, or they are blocked from covalent joining. The circularization ends of Claims 53-56 and 59-62 are between about 8 and about 50 nucleotides in length and upon hybridization are not substantially covalently joined by ligase or do not comprise cohesive ends of a bacteriophage. Support for Claims 51-64 can be found in the specification, for example, at Pages 3-5; Page 10, lines 16-31; Page 13, lines 14-26; Page 14, line 24 to Page 17, line 30; Page 21, lines 1-5; Page 21, line 19 to Page 22, line 25; Page 31, line 10 to Page 32, line 11; the Examples; and the accompanying Figures with the Descriptions to those Figures. Applicant submits that these Claims do not contain new matter.

Guegler discloses a cDNA library in pBluescript™ phagemid that was obtained by a mass excision of a cDNA library from the UNI-ZAP™ lambda phage (Stratagene, Inc.), using a f1 helper phage. As is known to one of skill in the art, such plasmid excision libraries comprise covalently closed circular DNA molecules.

In contrast, nucleic acid insert-vector constructs and libraries of Claims 51-53 and 57-60 are all linear with cohesive ends that either have a sequence which does not comprise that of a bacteriophage cohesive end or have attached enzymes or enzyme

complexes. Claim 63 is directed to linear genomic or cDNA library which has substantially no insert size bias relative to the population of genomic or cDNA molecules from which it was made. Applicant submits that nucleic acid inserts and genomic and cDNA libraries disclosed by Guegler provide no such features.

Similarly, Guegler provides no disclosure of the nucleic acid insert-vector constructs and libraries of Claims 55-56, 61-62 and 64, which are in circular vectors, nor of the insert-vector construct of Claim 54 which is linear. Claims 54-56 and 61-62 are directed to constructs and libraries which have two nicks at the site of joining the circularization ends and/or are not substantially covalently joined by ligase and/or do not comprise a bacteriophage cos site. Claim 64 is directed to circular genomic or cDNA library which has substantially no insert size bias relative to the population of genomic or cDNA molecules from which it was made. Applicant submits that the claimed inserts and libraries, which are in the present vectors, have not been disclosed by Guegler.

Applicant further submits that the novelty of Claims 51-64 resides in the products and not in the process of making those products as the Examiner has alleged. Guegler does not disclose cohesive ends that have a sequence which does not comprise that of a bacteriophage cohesive end. Guegler does not disclose cohesive ends that have attached enzymes or enzyme complexes. Guegler does not disclose nucleic acid inserts and libraries in circular vectors that have two nicks near the circularization site that are not substantially covalently joined by ligase. Nor does Guegler disclose libraries with substantially no insert size bias as is provided by the present invention. Applicant respectfully requests withdrawal of this rejection under 35 U.S.C. § 102(e).

Claims 31-34 and 37-39 have been rejected under 35 U.S.C. § 102(b) as

allegedly anticipated by Miki, 83 GENE 137-46 (1989) in light of MANIATIS, MOLECULAR CLONING, A LABORATORY MANUAL (Cold Spring Harbor Laboratory 1982). The Examiner has alleged that Miki teaches directional cloning of cDNA libraries by circularizing a λpCEV vector which allegedly has a cos site similar to the cohesive circularization ends of the present invention.

It is axiomatic that to establish that an invention is anticipated, a single reference must disclose each and every element of the claimed invention. *See, e.g., In re Donohue*, 766 F.2d 531, 226 U.S.P.Q. 619, 621 (Fed. Cir. 1985); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986). Applicant respectfully submits that this rejection is improper because two references are used by the Examiner to reject Claims 31-34 and 37-39 under 35 U.S.C. § 102(b) and requests withdrawal of this rejection.

Moreover, even if this rejection were made under 35 U.S.C. § 102(b) by citation only to Miki, this reference does not disclose the present invention because Miki is limited to a lambda-plasmid composite vector which must be packaged using the lambda packaging system.

As described above, Claims 31-32 have been canceled without prejudice and Claims 51-64 which contain some of the subject matter of Claims 31-32 have been added. Claim 33 has been amended to require that 90% of the nucleic acid fragments be inserted into circularized vectors. Support for this amendment to Claim 33 can be found, for example, at Page 20, Lines 14-18. Claim 65 has been added which contains some of the subject matter of Claim 33 and the limitation that the circularized vectors do not comprise a bacteriophage cos site. Support for this limitation can be found, for example, in Example 1. Claims 34-35 have

been amended to depend from Claim 65 as well as Claim 33. New Claim 66 has been added which depends from Claims 37-38 and is directed to a vector with cohesive circularization ends that do not comprise the cohesive ends of a bacteriophage. Claim 39 has been canceled without prejudice. Applicant submits that these amendments do not constitute new matter.

In contrast to the subject matter of Claims 33-34, 37-38 and 51-66, Miki is limited to disclosure of cloning procedures using a composite lambda-plasmid vector that has the entire sequence of a plasmid inserted into a Not I site of lambda phage. This means that, after cDNA insertion at a cloning site, the ligated lambda-plasmid-cDNA insert product must be packaged *in vitro* using the packaging system of lambda phage. Miki did not create a cDNA library in a plasmid vector, and only discloses excision of individual plasmids with a Not I restriction enzyme when those individual plasmids have inserts of interest. Hence, the only library provided by Miki is a lambda library that has already been packaged into phage particles.

The Examiner further has alleged that Miki teaches that hybridization of cohesive circularization ends (cos) is produced automatically due to the base-pairing specificity, indicating that ligase does not substantially covalently join the cohesive ends. Claims 37, 38, 54, 55, 60, 61 and 66 are directed *inter alia* to constructs wherein, upon hybridization, ligase does not substantially covalently join the cohesive circularization ends. Applicant respectfully submits that Miki is limited to a disclosure of cohesive circularization ends that *are* substantially covalently joined by ligase. In particular, Miki explicitly teaches that the cohesive ends of his lambda phage (λpCEV) vector are covalently sealed by ligase. *See* Miki at 139. According to Miki, the ligated λpCEV vector is then cut with restriction enzymes Sfi I and Eco RI, the resulting Sfi I ends are ligated to adaptors which then are

ligated with cDNA insert. This reaction produces concatemers of linear lambda genomes and inserts where the cohesive circularization ends are substantially covalently joined by ligase. It is known to one of skill in the art that lambda phage genomes are generated during the lytic growth by the rolling-circle replication which does not rely upon ligase -- instead it produces a long concatemers of the lambda phage genome (*see* SAMBROOK, MOLECULAR CLONING, A LABORATORY MANUAL at 2.5 (2d ed. 1989)). Lambda cos sites are cleaved again only during packaging of phage particles by the *ter* function of the A protein (*id*, p. 2.7). The lambda cos ends are hybridized and ligated only after the entry of a lambda phage particle into a bacterial cell (*id,* p. 2.4). Hence, Miki is limited to a disclosure of cohesive circularization ends that *are* substantially covalently joined by ligase, and does not disclose constructs explicitly designed to have cohesive circularization ends that are not substantially covalently joined by ligase.

The Examiner further has alleged that Miki teaches that all of the clones obtained contain single inserts in the defined orientation and that it is known that lambda phage circularizes upon entering the bacterial host as a linear DNA. Applicant submits that Miki discloses that "more than 90% of the clones contain inserts." *See* Miki Abstract. This statement does not address what proportion of the original insert population has been cloned into vector. In contrast, Claims 33, 34 and 65 recite that at least 90% of nucleic acid fragments are inserted into circularized vectors. It is well known in the art that the efficiency of packaging does not exceed about 10% (*see Lech, in* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 1987, p. 1.10.1). Therefore, according to conventional wisdom, only 10% of cDNA inserts of Miki are circularized into lambda – cDNA constructs. Hence, Miki does not satisfy the requirement of Claims 33, 34 and 65 that at least 90% of nucleic acid fragments

are inserted into circularized vectors.

Applicant submits Miki does not disclose cohesive ends which do not comprise those of bacteriophage cohesive ends. Accordingly, Claims 53, 56, 59, and 62 are not anticipated by Miki. Miki does not disclose cohesive ends that have attached enzymes or enzyme complexes as provided by Claims 51, 52, 57 and 58. Miki also does not disclose nucleic acid inserts and libraries in circular vectors that have two nicks near the circularization site as provided by Claims 53, 56, 59, and 62. Nor does Miki disclose libraries with substantially no insert size bias as is provided by Claims 63 and 64. Finally, Miki does not disclose the present nucleic acid inserts and genomic and cDNA libraries in vectors which require no packaging with the lambda packaging system. Accordingly, Applicant respectfully requests withdrawal of this rejection under 35 U.S.C. § 102(b).

Claim Rejection Under 35 U.S.C. § 103(a)

Claims 33-40 have been rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Miki, 83 GENE 137-46 (1989) in light of Aslanidis, 4 PCR METHODS AND APPLICATIONS 172-77 (Dec. 1994). The Examiner acknowledges that Miki does not explicitly teach the length of the nucleotide tail at the cohesive ends. However, according to the Examiner, Aslanidis teaches what is the minimal length required for single-stranded tails for ligation-independent cloning. Therefore, according to the Examiner, the mixture of Claim 33-34, the host cells of Claim 35, the vectors of Claims 36-39, and the kit of Claim 40 would have been obvious to one of ordinary skill in the art at the time this invention was made.

As described above, Claim 65 has been added which contains some of the subject matter of Claim 33 and the limitation that the circularized vectors do not comprise a bacteriophage cos site. Support for this limitation can be found, for example, in Example 1.

Claims 34-35 have been amended to depend from Claim 65 as well as Claim 33. New Claim 66 has been added which depends from Claims 37 and 38 and is directed to a vector with cohesive circularization ends that do not comprise the cohesive ends of a bacteriophage. Claim 39 has been canceled without prejudice. Applicant submits that these amendments do not constitute new matter.

Applicant submits that even if it does teach minimal requirements for single-stranded tails in ligation-independent cloning, Aslanidis does not cure the limitations of Miki described above.

Miki is limited *inter alia* to disclosure of cloning procedures using a lambda-plasmid composite vector that has the entire sequence of a plasmid. Miki therefore provides no alternative to *in vitro* bacteriophage packaging for creating a library of cDNA or genomic DNA fragments. It is well known in the art that the efficiency of packaging does not exceed about 10% (*see Lech, in* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 1987, p. 1.10.1). Neither Miki nor Aslanidis teach what proportion of the original insert population has been cloned into vector. Given the teachings of Lech, only 10% of cDNA inserts of Miki are circularized into lambda – cDNA constructs. While Aslanidis states at page 172 that "[t]he procedure seems to result in 100% recombinant clones," this statement merely teaches that inserts were cloned into almost 100% of the circularized vectors. This statement does not teach whether 90% or more of the original insert population was cloned. Hence, the combination of Miki with Aslanidis does not satisfy the requirement of Claims 33-36 and 65 that at least 90% of nucleic acid fragments are inserted into circularized vectors.

Moreover, Aslanidis does not teach independent insertion and circularization sites nor cohesive circularization ends which cannot be substantially covalently joined by ligase, as

provided in Claims 37, 38, 40 and 66. As depicted in Figure 1 of Aslanidis, the insertion and circularization sites are identical and the hybridized ends of the insert and vector have 5'-phosphates, indicating that these hybridized ends could easily be ligated.

Applicant respectfully requests withdrawal of this rejection under 35 U.S.C. § 103(a).

Conclusion

It is believed that the present application is in a condition for allowance which action is earnestly solicited. If the Examiner has any questions, the undersigned can be contacted to discuss this application.

Respectfully submitted,

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